Genetic variants in the *ADAMTS13* and *SUPT3H* genes are associated with ADAMTS13 activity

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Statement of equal author contribution

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- We identify rs41314453 as the strongest genetic predictor of ADAMTS13 activity, associated with a decrease of over 20%.

- We present evidence of further independent associations with a common variant in SUPT3H, as well as 5 variants at the ADAMTS13 locus.
Abstract

ADAMTS13 cleaves von Willebrand factor, reducing its prothrombotic activity. The genetic determinants of ADAMTS13 activity remain unclear. We performed a genome-wide association study of ADAMTS13 activity in the Rotterdam Study, a population-based cohort study. We used imputed genotypes of common variants in a discovery sample of 3,443 individuals and replication sample of 2,025 individuals. We examined rare exonic variant associations in ADAMTS13 in 1,609 individuals using an exome array. rs41314453 in ADAMTS13 was associated with ADAMTS13 activity in both our discovery (Beta: -20.2%, P-value: 1.3×10^{-33}) and replication sample (P-value: 3.3×10^{-34}), and explained 3.6-6.5% of the variance. In the combined analysis of our discovery and replication samples, there were two further independent associations at the ADAMTS13 locus: rs3118667 (Beta: 3.0, P-value: 9.6×10^{-21}) and rs139911703 (Beta: -11.6, P-value: 3.6×10^{-8}). Additionally, rs10456544 in SUPT3H was associated with a 4.2 increase in ADAMTS13 activity (P-value: 1.13×10^{-8}). Finally, we found three independent associations with rare coding variants in ADAMTS13: rs148312697 (Beta: -32.2%, P-value: 3.7×10^{-6}), rs142572218 (Beta: -46.0%, P-value: 3.9×10^{-5}), and rs36222275 (Beta: -13.9%, P-value: 2.9×10^{-5}). In conclusion, we identified rs41314453 as the main genetic determinant of ADAMTS13 activity, and present preliminary for further associations at the ADAMTS13 and SUPT3H loci.

Key words: ADAMTS13, von Willebrand factor, thrombosis, genetics, coagulation, platelets
Introduction

ADAMTS13 (A Disintegrin And Metalloproteinase with ThromboSpondin motifs 13) cleaves ultra large von Willebrand Factor (VWF) into smaller multimers.\textsuperscript{1-3} ADAMTS13 thereby greatly reduces the activity of VWF in its role in platelet adhesion and aggregation. Through this effect on VWF, ADAMTS13 has antithrombotic properties.

The role of ADAMTS13 in thrombosis is especially evident in patients with thrombocytopenic thrombotic purpura (TTP), a disorder resulting from a severe deficiency of ADAMTS13: patients with TTP have a wide range of symptoms, including thrombocytopenia and microangiopathy, which may result in stroke, and myocardial infarction.\textsuperscript{4} Beyond patients with TTP, we and others recently showed that low ADAMTS13 activity and levels within the normal range are also associated with increased risk of cardiovascular outcomes.\textsuperscript{5-9}

These associations between ADAMTS13 activity and arterial thrombosis raise the question of how ADAMTS13 activity is regulated. Several rare single nucleotide polymorphisms (SNPs) in the \textit{ADAMTS13} gene causing TTP have been identified along with a few common variants with more modest effects on ADAMTS13.\textsuperscript{10,11} However, it is not known whether these associations are independent of each other, or even whether they exhibit the strongest associations at the locus. Furthermore, the role of genetic variation outside of the ADAMTS13 locus remains unknown. The optimal method to identify genetic determinants is a genome-wide association (GWA) study, with a hypothesis-free approach. To date, no studies on the genetics of ADAMTS13 using this approach have been reported.

Thus, in the Rotterdam Study, a large population-based cohort study, we conducted a GWA study of ADAMTS13 activity, including a conditional analysis to identify multiple
independent signals. Additionally, we characterized the ADAMTS13 gene and any other genes with associated common variants by examining the role of rare variants.

Methods

Study description and population

The Rotterdam Study is a prospective, population-based cohort study of determinants of several chronic diseases in older adults. The first cohort (RS-I), includes 7,983 inhabitants of Ommoord, a district of Rotterdam in the Netherlands, who were 55 years or older. The baseline examination took place between 1990 and 1993. The third visit took place between March 1997 and December 1999, and included 4,797 participants. A second cohort (RS-II) was established between February 2000 and December 2001, including another 3,011 inhabitants of Ommoord who reached the age of 55 years after the baseline examination of RS-I, and individuals aged 55 years or older who had migrated into the research area. The study was approved by the Medical Ethics Committee of Erasmus University, Rotterdam, the Netherlands, and all included participants gave their written informed consent in accordance with the Declaration of Helsinki.

ADAMTS13 measurement

Citrated plasma samples were collected at the third visit of RS-I and the baseline examination of RS-II, and stored at -80°C. Between June and October 2013, we measured ADAMTS13 activity using a kinetic assay based on the Fluorescence Resonance Energy Transfer Substrate VWF 73 (FRETS-VWF73) assay. This assay uses a peptide containing the ADAMTS13 cleavage site of VWF, and thus captures variation in the VWF cleavage rate determined by ADAMTS13 levels and structure, but not by alterations in VWF.
Plasma samples were measured against a reference curve of serial dilutions of normal human plasma defined to have an ADAMTS13 activity of 1 IU/ml, and we express ADAMTS13 activity as a percentage of this. In total, the ADAMTS13 activity of 6,258 participants was measured: 3,791 from RS-I, and 2,467 from RS-II.

Genotyping and imputation

We used two sources of genetic variants: genome-wide SNPs genotyped by the Illumina Infinum II HumanHap550 array or 610 quad array and exome-wide SNPs genotyped by the Illumina HumanExome BeadChip v1.0. We genotyped 6,291 participants from RS-I and 2,157 participants from RS-II using the Illumina Infinium II HumanHap550 or 610 quad arrays. All genotyped participants were of European ancestry based on their self-report. Prior to imputation, genotyped SNPs with a call rate below 98%, a minor allele frequency (MAF) below 1%, or a hardy-weinberg equilibrium P-value of less than $1 \times 10^{-6}$ were excluded. In RS-I 512,849 SNPs remained after filtering and these were used for imputation. In RS-II, 537,405 SNPs were used for imputation. Dosages of 19,537,258 SNPs were imputed in both studies using the Genomes of the Netherlands (GoNL) version 4 reference panel. MACH version 1.0.15 was used to perform the imputations. The imputation quality of each SNP defined as the estimated squared correlation of imputed and true genotypes, and ranged from 0 to 1. After imputation, SNPs with a MAF below 0.01 or an imputation quality below 0.3 were excluded. The overlap between participants with ADAMTS13 activity measurements and genotypes was 3,423 in RS-I, and 2,025 in RS-II.
Exonic variants of 3,163 individuals from RS-I were successfully genotyped using the Illumina HumanExome BeadChip v1.0. In 1,609 of these individuals ADAMTS13 was measured. Genotype calling was performed at the University of Texas Health Science Center in Houston, together with ten other cohorts from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium. This joint calling in a total of 62,000 individuals was done to improve the calling of rare variants compared to what could be accomplished in RS-I alone. A total of 108,678 SNPs were included after filtering out monomorphic SNPs and SNPs with low call rate.

**Common variant analysis**

We performed a discovery GWA analysis in RS-I. In the discovery, linear regression, as implemented in ProbABEL version 0.4.3, was used to examine the association of each SNP with ADAMTS13 activity, adjusted for age and sex. SNPs were analysed in the form of genotype dosages (ranging from 0 to 2) using an additive model. A genome-wide significance threshold of $5 \times 10^{-8}$ was used. Regional plots were created using locuszoom.

Replication analyses in RS-II were also performed using ProbABEL version 0.4.3. The significance threshold was determined using a Bonferroni correction for the number of SNPs. The variance of ADAMTS13 activity explained by replicating SNPs was examined, with R version 3.1.1. We used HaploReg V2 to browse ENCODE resource to examine the functional implication of these SNPs, along with any correlated SNPs (Correlation R2 > 0.8).

Lastly, to maximize our power and the accuracy of our effect estimates, we also performed a meta-analysis of RS-I and RS-II. We used an inverse-variance model with fixed effects as implemented in METAL. We applied a genomic control correction to the combined
results to account for genomic inflation. To identify secondary signals at significant loci, we then performed a stepwise conditional analysis repeating the GWA analysis adjusted for the most significant variant in each locus (defined as +/- 250KB of the top SNP). This approach was repeated with additional adjustment for secondary signals until no further genome-wide significant signals remained.

**Rare variant analysis**

In a subset of RS-I participants, we used the exome chip to examine the effect of rare variants. To maximize our power, we included only SNPs within genes that were highlighted in the common variant analysis. Additionally, we only included SNPs that were functional according to the dbNSFP database (missense, stop-gain, stop-loss or splice site) with a MAF below 0.01. We then used the seqMeta package implemented in R to determine the association between the rare variant burden in selected genes and ADAMTS13 activity, and to examine the association of individual SNPs. This package has previously been described in further detail. Rare variant burden analysis was performed using both a T1 test, and a sequence kernel association test (SKAT). In T1 tests, the sum of rare variant dosages is created for each gene, and associated with the traits of interest. T1 tests are unidirectional: they are more powerful when, within a gene, the effect sizes of rare variants are consistently in the same direction. SKAT is a bidirectional test and is more powerful when the effect direction of rare variants within a gene varies. Single variant analysis was done using score tests. All analyses were adjusted for age, sex, and the independently significant common variants. Additionally, the analyses were adjusted for four ancestry-informative principal components, as rare variants are more susceptible population stratification. Finally,
we performed stepwise conditional analysis to determine whether rare variant associations were independent from each other.

**Estimation of the heritability**

In RS-I, we estimated the proportion of variance of ADAMTS13 activity explained by all SNPs together. First, we constructed a matrix of pairwise genetic relationships based on common (MAF ≥ 0.01) well-imputed (imputation quality > 0.3) SNPs. We excluded one individual from each pair with a pairwise relationship larger than 0.025, reducing the number of included individuals to 2455. We then used a restricted maximum likelihood model to estimate the proportion of variance explained by the genetic relationships. The result can be interpreted as the lower bound of the heritability.\(^{28}\) The estimated heritability is expected to be lower than the true heritability because it is based on imperfectly imputed SNPs that may in turn be only partially correlated to the underlying causal variants.

We then calculated the variance explained by the combination of independently significant variants using the adjusted R-squared resulting from a linear regression model in R. We did this separately for RS-I and RS-II.

Additionally, to place genetic determinants of ADAMTS13 into a wider context, we estimated the variance of ADAMTS13 activity explained by genome-wide significant SNPs, as well as by baseline characteristics including age, sex, total and high density lipoprotein (HDL) cholesterol, prevalent type 2 diabetes, current smoking, body mass index (BMI), and systolic and diastolic blood pressure. We used the partial.R2 function from the asbio package in R. All variables were included in a single linear regression model, and the resulting partial coefficients of determination indicate the variance explained on top of the other variables in the model.
Results

Discovery in RS-I and Replication in RS-II

Participant characteristics are shown in Table 1. Participants in RS-I were older (mean age = 72.4 years old, standard deviation = ±7.0) than participants in RS-II (mean age = 64.6 years old, standard deviation = ±7.9). The mean ADAMTS13 was 89.5% in RS-I and 95.0% in RS-II, with a range of 5% to 198% across the two cohorts. After removing rare and poorly imputed SNPs, 8,237,900 SNPs were included in the discovery GWA analysis, of which 329 were significantly associated with ADAMTS13 activity (Supplementary Figure 1 and 2). All of these SNPs mapped to the ADAMTS13 locus. The minor allele of the lead SNP, rs41314453, was associated with a 20.2% decrease in ADAMTS13 activity ($P$-value = 1.3×10^{-33}). The signal was successfully replicated in RS-II: the minor allele of rs41314453 was associated with a 23.5% decrease in ADAMTS13 activity ($P$-value = 3.3×10^{-34}).

Combined analysis of RS-I and RS-II

In the combined analysis of RS-I and RS-II rs41314453 was also the lead variant at the ADAMTS13 locus (Table 2 and Figure 1A). There was one genome-wide significant SNP outside of the ADAMTS13 locus: rs10456544, an intronic SNP in the SUPT3H gene (Table 2 and Figure 1B). The minor allele was associated with a 4.2% increase in ADAMTS13 activity. After adjustment for rs41314453 and rs10456544, there were no significant variants remaining at the SUPT3H locus, but there was a second signal at the ADAMTS13 locus. The minor allele of lead variant rs3118667 was associated with 3.0% increase in ADAMTS13 activity (Table 2). When additionally adjusting for rs3118667, there was a third genome-wide significant signal at the
ADAMTS13 locus. The minor allele of the lead variant, rs139911703, was associated with an 11.6% decrease in ADAMTS13 activity (Table 2).

**Rare variant analyses**

There were 11 functional SNPs with MAF < 0.01% in ADAMTS13 and 4 in SUPT3H. For single variant analysis, we thus used a $P$-value threshold of 0.0033. Three rare variants were associated with ADAMTS13 activity: rs148312697 ($\text{Beta} = -32.8$, $P$-value = $3.6 \times 10^{-6}$, Frequency = 0.16%), rs142572218 ($\text{Beta} = -46.0$, $P$-value = $3.9 \times 10^{-5}$, Frequency = 0.06%), and rs36222275 ($\text{Beta} = -14.7$, $P$-value = $2.2 \times 10^{-3}$, Frequency = 0.34%). The association of these variants was independent of the three associated common variants in ADAMTS13 (Table 3), and stepwise conditional analysis suggests that the associations are also independent of each other (Supplementary Table 1). The spread across the functional domains of ADAMTS13 of these associated rare nonsynonymous variants, as well as the associated common nonsynonymous variant (rs41314453), is shown in Figure 2. None of the rare variants in SUPT3H was significantly associated to ADAMTS13 activity.

Although we only examined two genes, we used a $P$-value threshold of 0.013 to adjust for doing both SKAT and T1 tests. The 11 variants in ADAMTS13 had a cumulative minor allele frequency of 1.1%. Rare variant burden in ADAMTS13 was associated with ADAMTS13 activity according to both the T1 ($P$-value = $5.7 \times 10^{-8}$) and SKAT test ($P$-value = $1.5 \times 10^{-6}$). These associations remained significant after adjusting for the three associated common variants in ADAMTS13 (Supplementary Table 2). When we additionally adjusted the burden tests for the three rare SNPs in a stepwise manner, the association diminished with each step, and finally lost significance upon adjustment for all three rare SNPs (Supplementary Table 2). The rare variant
burden in *SUPT3H* was not associated to ADAMTS13 activity according to the T1 (*P*-value = 0.5) and SKAT tests (*P*-value = 0.7).

*Estimation of the heritability*

The variance of ADAMTS13 activity explained by all SNPs in RS-I was 35.2% (*P*-value = 0.009), which can be interpreted as the lower bound of the heritability. The variance explained by the four independently significant common SNPs was 5.8-8.2%. The variance of ADAMTS13 activity explained by each of the four independently significant common SNPs on top of other baseline characteristics is shown in Supplementary Table 3. This table also shows the variance explained by other baseline characteristics. The variance explained by rs41314453 (3.6-6.5%) is comparable to the variance explained by age (3.9-6.5%) as well as the variance explained by sex (4.5-6.7%). The variance explained by rs3118667 (1.3-2.1%) is comparable to the variance explained by current smoking (1.5-1.7%). Because the estimates for SNPs are based on imputed dosages rather than directly measured genotypes, the actual variance explained by the SNPs is likely to be higher.

**Discussion**

In this first-ever GWA study of ADAMTS13 activity, we robustly identified rs41314453 within the *ADAMTS13* gene as the main genetic determinant of ADAMTS13 activity in both our discovery and replication cohort, explaining between 3.6 and 6.5 percent of the variance. Through the combined analysis of our discovery and replication samples, we present preliminary evidence of independent associations with two further SNPs in *ADAMTS13* (rs3118667 and rs139911703), and with a SNP in the *SUPT3H* gene (rs10456544). Furthermore, in a subset of
our discovery sample, we found 3 independently associated rare variants in \textit{ADAMTS13} (rs148312697, rs142572218, and rs36222275). Finally, we established a lower bound for the heritability of ADAMTS13 activity at 35%.

The most significant SNP, rs41314453, is a nonsynonymous exonic variant in the thrombospondin type 1 repeat 2 domain that is also known as Ala732Val. It is in linkage disequilibrium with several intronic SNPs in \textit{ADAMTS13}, as well as to SNPs in regulatory regions of neighbouring genes. However, rs41314453 remains the most promising candidate causal SNP, because it has previously been shown, \textit{in vitro}, to reduce ADAMTS13 levels by 40% and ADAMTS13 activity by 29%.\(^{29}\) The decrease in activity appeared to be mediated completely by the decrease in protein concentration rather than a decrease in the specific activity (activity per milligram of ADAMTS13), and the decrease in levels was not linked to decreased synthesis.\(^{29}\) This suggests that the underlying mechanism is a decreased secretion of ADAMTS13.

The secondary signal at the \textit{ADAMTS13} locus, rs3118667, is a synonymous SNP that has not previously been reported to be associated with ADAMTS13. It is not in strong linkage disequilibrium with other SNPs. Thus, the mechanism behind this signal is unclear. The third signal at the \textit{ADAMTS13} locus, rs139911703, is an intronic SNP in \textit{OBP2B}. It is not strongly correlated to any variant in the \textit{ADAMTS13} gene, but it is in perfect linkage disequilibrium with rs36218903, an intronic variant in \textit{ABO}. The underlying mechanism may thus involve the \textit{ABO} gene although we cannot exclude an effect on the regulation of the \textit{ADAMTS13} gene, or correlation with an unknown coding variant. It is unclear how \textit{ABO} could regulate ADAMTS13 activity. Variation in the glycan structures attached to VWF that are encoded by the \textit{ABO} gene has been linked to the cleavage rate: cleavage was faster with VWF originating from individuals
However, this effect on the cleavage rate is not reflected in the ADAMTS13 activity measurements in this study, as the measurements are based on an introduced peptide spanning the VWF cleavage site.

Only one SNP outside of the \textit{ADAMTS13} locus was associated with ADAMTS13 activity: rs10456544 in \textit{SUPT3H}, which encodes the protein Spt3. As a part of the SPT3-TAF9-GCN5 acetyltransferase (STAGA) complex, Spt3 is involved in transcription activation. The STAGA complex acetylulates histones, reconfiguring the DNA around the histones into a more accessible structure, allowing for increased transcription. In yeast, around 3\% of the genome is dependent on Spt3 for expression. The main role of the Spt3 subunit in STAGA is to recruit the transcription factor II D complex (TFIID), which then binds to TATA box motifs in promoters, enabling RNA polymerase II to position itself appropriately for transcription. The \textit{ADAMTS13} promotor does not have a known TATA box motif, but it does have an Sp1 binding site, which can allow TFIID to bind to TATA-less promoters. We thus hypothesize that rs10456544 is associated to ADAMTS13 activity through a disturbance to these basal transcription activation processes. As ADAMTS13 does not appear to be heavily regulated by transcription factors, the sensitivity to these processes might be increased. The possible relationship between Spt3 and ADAMTS13 activity should be confirmed through replication of the association and functional work.

Of the three associated rare SNPs, rs148312697 (Asp187His), located in the metalloprotease domain, has been shown in mice to reduce ADAMTS13 activity and secretion and to cause TTP. Another variant at the same position (Asp187Ala) has also been shown to reduce proteolytic function. rs142572218 (Arg1060Trp) has been identified as a causal
mutation for late-onset adult TTP, and has been shown to profoundly decrease secretion, but not the specific activity.\textsuperscript{41} rs36222275 (Gly982Arg) has not previously been associated to ADAMTS13 activity. The effect size is smaller than that of the other two rare variants and rs41314453, the lead common variant. We were able to identify this rare variant with an intermediate effect size because of our hypothesis driven approach, but it will need to be confirmed either in vitro or through replication in other association studies.

Nonsynonymous variant rs28647808, or Pro618Ala, has previously been used as a genetic proxy of ADAMTS13 activity.\textsuperscript{42} Indeed, several lines of experimental evidence support a causal role for Pro618Ala.\textsuperscript{29,43} In the combined analysis of our discovery and replication samples, Pro618Ala was well-imputed (imputation quality > 0.9), and was associated with ADAMTS13 activity (Beta = -4.5, \( P \)-value = 7.3\( \times \)10\textsuperscript{-16}, Frequency = 9.8\%). However, this association disappeared after adjusting for the lead variant, rs41314453, with which it is in modest linkage disequilibrium (\( R^2 = 0.18 \)). In line with our results, studies by Miyata et al and Kokame et al have found no association between Pro618Ala and ADAMTS13 activity in the Japanese general population.\textsuperscript{43,44} Our results therefore do not support a causal role of rs28647808 in the regulation of ADAMTS13 activity, and suggest that rs41314453 may be a more suitable genetic proxy for future studies.

Similarly, another polymorphism that has been associated to ADAMTS13 activity in the literature,\textsuperscript{44} rs2301612 or Gln448Glu, was not strongly associated with ADAMTS13 activity in our study (Beta = 1.6, \( P \)-value = 1.4\( \times \)10\textsuperscript{-6}, Frequency = 43.6\%). The effect direction was consistent with the literature. Interestingly, the association became stronger upon adjustment for \textit{ADAMTS13} lead variant rs41314453 (Beta = 2.6, \( P \)-value = 1.1\( \times \)10\textsuperscript{-15}), but was again attenuated when further adjusted for secondary variant rs3118667 (Beta = 1.3, \( P \)-value = 1.4\( \times \)10\textsuperscript{-3}).
In the discovery GWA analysis, we only found associations with SNPs within the 
\textit{ADAMTS13} gene itself. In the combined analysis of the discovery and replication samples only 
one SNP at another locus was genome-wide significant. While this is likely related to the small 
sample size, the unbalanced genetic architecture is not surprising. ADAMTS13 is constantly 
synthesized and secreted in its active form. Previous work suggests that ADAMTS13 
transcription is stable and not significantly regulated by transcription factors.\textsuperscript{37} This leaves little 
room for strong regulators. Furthermore, while several factors are known to influence the rate at 
which ADAMTS13 cleaves VWF, these are not captured by the measurement of ADAMTS13 
activity. The measurement is based on the rate at which an introduced peptide similar to VWF is 
cleaved. However, \textit{in vivo}, alterations to VWF that disrupt its interactions with ADAMTS13 may 
also affect the cleavage rate. For example, mutations involved in type 2A von Willebrand disease 
have been shown to increase the cleavage rate.\textsuperscript{45}

Apart from synthesis and secretion, ADAMTS13 activity is further determined by 
degradation, and the specific activity. ADAMTS13 degradation is known to occur in the 
presence of thrombin and plasmin.\textsuperscript{46} However, the level of ADAMTS13 degradation is minimal, 
since coagulation and fibrinolysis are normally only occurring at a very low level. We therefore 
expect the regulation of ADAMTS13 degradation to explain a very small part of the genetic 
associations with ADAMTS13 activity.

In patients with congenital ADAMTS13 deficiency, who often suffer from TTP, the main 
underlying mechanisms are changes in secretion and specific activity.\textsuperscript{11} This is in line with our 
results in this population based study. Functional work has previously been done for three of the 
variants associated with ADAMTS13 activity in our study, and two of these reduce secretion, 
while one reduces the specific activity.\textsuperscript{29,40,41}
The strengths of this study include our genome-wide hypothesis-free approach, which, in contrast to the targeted genotyping of a few candidate SNPs, allowed us to systematically examine the ADAMTS13 locus. Secondly, the use of GoNL as a reference panel for the imputation of unmeasured SNPs was particularly appropriate, as this reference panel is based specifically on the Dutch population. Thirdly, we were able to replicate our common variant results in a non-overlapping sample that was ethnically similar to the discovery sample and used the same assay to measure ADAMTS13 activity. Finally, the rare variant and conditional analyses we performed allowed us to gain a detailed view of the ADAMTS13 locus.

However, while two of the rare variant associations were backed up by previous functional work, we were not able to replicate our rare variant associations because participants in RS-II were not genotyped using the exome chip. Neither were we able to replicate the associations with rs3118667 and rs139911703 in ADAMTS13 nor rs10456544 in SUPT3H, as these associations were identified by combining our discovery and replication samples. Additionally, the limited sample size allowed us to detect only the strongest associations with ADAMTS13 activity. This will be improved as more studies with genome-wide SNP array data measure ADAMTS13 activity or levels. Although we replicated our results in a non-overlapping sample, both samples were from the Rotterdam Study and were measured together. Thus, the samples were not completely independent from one another. Finally, our estimate of the heritability should be interpreted as the lower bound of the heritability for two reasons. First, it is based on imperfectly imputed SNPs that may in turn be only partially correlated to the underlying causal variants. Second, it is only based on common SNPs, while a portion of the heritability is likely to stem from rare variants. Estimates from twin and family studies are required for further precision.
In conclusion, in our study we robustly identified a strong association between rs41314453 in the \textit{ADAMTS13} gene and ADAMTS13 activity, and we present preliminary evidence of association with another five genetic variants in \textit{ADAMTS13} and one variant in the \textit{SUPT3H} gene. Explaining between 3.6 and 6.5 percent of the variance, rs41314453 appears to be the main genetic determinant of ADAMTS13 activity.

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O.H. Franco works in ErasmusAGE, a center for aging research across the life course funded by Nestlé Nutrition (Nestec Ltd.), Metagenics Inc., and AXA.

Author contributions
P. de Vries, F. Leebeek, O. Franco, M. de Maat, and A. Dehghan designed the research. F. Rivadeneira, M. Ikram, H. Rottensteiner, A. Hofman and A. Uitterlinden collected data. J. Boender and M. Sonneveld analyzed and interpreted data. P. de Vries performed statistical analysis. P. de Vries, M. de Maat, and A. Dehghan wrote the manuscript. All authors were involved in the revision of the manuscript.

Conflicts of interest
H. Rottensteiner is a full time employee of Baxter Innovations GmbH, Vienna, Austria. O.H. Franco works in ErasmusAGE, a center for aging research across the life course funded by Nestlé Nutrition (Nestec Ltd.), Metagenics Inc., and AXA. Nestlé Nutrition (Nestec Ltd.), Metagenics Inc., and AXA had no role in the design and conduct of the study, the collection, management, analysis, and interpretation of the data, nor in the preparation, review or approval of the manuscript.
References

Tables

Table 1: Characteristics of the participants included in the discovery in the Rotterdam Study I (RS-I) and in the replication in the Rotterdam Study II (RS-II).

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<td>ADAMTS13 activity (%)</td>
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</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.8 ±1.0</td>
<td>5.8 ±1.0</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.4 ±0.4</td>
<td>1.4 ±0.4</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>143.3 ±21.0</td>
<td>143.1 ±21.3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.2 ±11.2</td>
<td>78.9 ±10.8</td>
</tr>
<tr>
<td>Prevalent Type 2 Diabetes (%)</td>
<td>14.1</td>
<td>11.5</td>
</tr>
</tbody>
</table>

Continuous variables are summarized by their mean ± standard deviation. Abbreviations: BMI refers to body mass index, and HDL refers to high-density lipoprotein.
### Table 2: Association of common variants with ADAMTS13 activity in the combined analysis of RS-I and RS-II.

<table>
<thead>
<tr>
<th>SNP Name</th>
<th>Chromosome</th>
<th>Position</th>
<th>Gene</th>
<th>Effect / Other Allele</th>
<th>Frequency</th>
<th>Imputation Quality</th>
<th>Beta</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs41314453</td>
<td>9</td>
<td>136,307,825</td>
<td>ADAMTS13</td>
<td>T/C</td>
<td>1.88%</td>
<td>0.84</td>
<td>-21.7</td>
<td>1.2×10⁻⁶³</td>
</tr>
<tr>
<td>rs10456544</td>
<td>6</td>
<td>45,181,694</td>
<td>SUPT3H</td>
<td>A/T</td>
<td>7.11%</td>
<td>0.69</td>
<td>4.2</td>
<td>1.1×10⁻⁸</td>
</tr>
</tbody>
</table>

*Additional adjustment for rs41314453 and rs10456544*

| rs3118667    | 9          | 136,291,063| ADAMTS13 | C/T                   | 47.09%    | 0.93               | 3.0      | 9.6×10⁻²¹   |

*Additional adjustment for rs3118667*

| rs139911703  | 9          | 136,081,887| OBP2B    | A/G                   | 1.10%     | 0.52               | -11.6    | 3.6×10⁻⁸    |

**Abbreviations:** SNP refers to single nucleotide polymorphism. Frequency refers to the frequency of the effect allele as a percentage.

Beta refers to the beta coefficient, and should be interpreted as the change in ADAMTS13 activity (%) per 1 allele increase in the effect allele. *The DNA position is coded according the build 37.*
**Table 3:** Association of rare non-synonymous exonic variants in the *ADAMTS13* gene with ADAMTS13 activity, adjusted for common variants rs41314453, rs3118667, and rs139911703.

<table>
<thead>
<tr>
<th>SNP Name</th>
<th>Amino Acid Change</th>
<th>Position*</th>
<th>Exon</th>
<th>Effect / Other Allele</th>
<th>Frequency</th>
<th>Beta</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs148312697</td>
<td>Asp187His</td>
<td>136,291,338</td>
<td>6</td>
<td>C/G</td>
<td>0.16%</td>
<td>-32.1</td>
<td>3.3×10^{-6}</td>
</tr>
<tr>
<td>rs142572218</td>
<td>Arg1060Trp</td>
<td>136,319,670</td>
<td>24</td>
<td>T/C</td>
<td>0.06%</td>
<td>-46.7</td>
<td>1.8×10^{-5}</td>
</tr>
<tr>
<td>rs36222275</td>
<td>Gly982Arg</td>
<td>136,314,986</td>
<td>23</td>
<td>A/G</td>
<td>0.34%</td>
<td>-13.3</td>
<td>4.4×10^{-1}</td>
</tr>
</tbody>
</table>

*Abbreviations:* SNP refers to single nucleotide polymorphism. Frequency refers to the frequency of the effect allele. Beta refers to the beta coefficient, and should be interpreted as the change in ADAMTS13 activity (%) per 1 allele increase in the effect allele. *The DNA position is coded according the build 37, and refers to the position on chromosome 9.

*Adjustments:* Age, sex, principal components 1-4, rs41314453, rs3118667, and rs139911703.
Figure Legends

Figure 1: Regional plots of the association between ADAMTS13 activity and A) the ADAMTS13 locus and B) the SUPT3H locus in the combined GWA analysis. Linkage disequilibrium of variants is shown with A) rs41314453, B) rs10456544.

Figure 2: Location of the independently associated nonsynonymous variants across the functional domains of ADAMTS13.

Asp187His is rs148312697, Ala732Val is rs41314453, Gly982Arg is rs36222275, and Arg1060Trp is rs142572218. Thrombospondin type 1 repeats 1-8 are shown as circles. Cys-rich indicates the cysteine rich domain, and CUB indicates the C1r-C1s, urinary epidermal growth factor, and bone morphogenetic protein domains.
Figure 1

A) 

B)
Figure 2
Genetic variants in the ADAMTS13 and SUPT3H genes are associated with ADAMTS13 activity


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